Eicosanoid synthesis in duodenal ulcer disease: decrease in leukotriene C4 by colloidal bismuth subcitrate

A Ahmed, P R Salmon, C R Cairns, M Hobsley, J R S Hoults

Abstract

The release of immunoreactive prostaglandin E2 (PGE2) and leukotriene C4 (LTC4) from antral and duodenal mucosal biopsy specimens taken from 20 patients with duodenal ulcer disease was measured by radioimmunoassay before and four weeks after treatment with colloidal bismuth subcitrate. Gastroscopic and histological examination showed complete ulcer healing in 15/18 patients and duodenal histology looked normal (n=15) or improved (n=3); two patients failed to attend for a second endoscopy. Analysis of the supernatant from incubations of biopsy tissue in vitro showed that unstimulated antral release of PGE2 was significantly more than that from the duodenal mucosa (p<0.05), whereas basal release of LTC4 was significantly lower from antral biopsy specimens (p<0.05). Subsequent incubation of specimens with calcium ionophore A23187 caused an increase in LTC4 but not in PGE2 generation. The ability of antral and duodenal mucosa to form ionophore mediated LTC4 in patients with duodenal ulcer disease was significantly greater (p<0.05; p<0.01 respectively) than that of normal gastroduodenal mucosa. After colloidal bismuth subcitrate treatment, basal synthesis of PGE2 was unchanged in duodenal and antral specimens. In contrast, basal duodenal LTC4 was reduced (p<0.05), and the capacity for ionophore mediated duodenal LTC4 formation was substantially and significantly reduced after treatment (p<0.001). These results indicate that after therapeutic healing of duodenal ulcer (accompanied by clearance of inflammatory cell infiltrate), there is a reduced ability of duodenal mucosa to generate proinflammatory peptidoleukotrienes.

The role of prostaglandins in the pathogenesis of duodenal ulcer disease remains controversial, and the importance of the vasconepressor and proinflammatory peptidoleukotriene LTC4 is not fully established. Early reports showed that exogenous LTC4 exerts a powerful influence on the rat gastric microcirculation leading to a reduction in mucosal blood flow, and that mucosal generation of LTC4 in rats was increased considerably after ethanol administration. The initial findings of Peskar et al10 and Wallace et al11 that inhibitors of peptidoleukotriene synthesis and the dual cyclo-oxygenase/5-lipoxygenase inhibitor BW 755C offer appreciable protection against ethanol induced damage in rats remains controversial. Boughton-Smith et al, however, have shown that the selective 5-

lipoxygenase inhibitor BWA4C affords partial protection against platelet activating factor (PAF) induced gastric damage. Moreover, infusion of LTC4 increases the susceptibility of the rat stomach to injury by ethanol.12

Colloidal bismuth subcitrate is known to be effective in promoting duodenal ulcer healing in man13 and seems to offer the prospect of a lower rate of relapse than follows successful treatment with histamine H2-receptor antagonists.14 Although the mechanism of these effects has not been fully explained, animal studies show that colloidal bismuth subcitrate is protective against ethanol induced gastric lesions and that this is associated with increased mucosal generation of prostaglandin E2 (PGE2).15 Recent studies have shown that colloidal bismuth subcitrate provides partial protection from aspirin induced lesions despite concomitant suppression of prostaglandin biosynthesis.1617 This suggests that it may confer protection by mechanisms other than by increasing prostaglandin production.

There is a need for further investigation of the relation between colloidal bismuth subcitrate, ulcer healing, and arachidonate metabolism in man. In this study the release of immunoreactive LTC4 and PGE2 from human gastroduodenal mucosal biopsy tissues was measured ex vivo in patients with duodenal ulcer disease both before and four weeks after a successful course of colloidal bismuth subcitrate treatment.

Methods

Patients

Twenty patients (15 men and five women), mean age 46 (range 19–77) years with an endoscopically proved duodenal ulcer and 15 subjects with upper abdominal pain who were found to be endoscopically normal (12 men and three women), mean age 42 (range 22–69) years were studied. Endoscopic biopsy specimens were taken before treatment and, in the case of the duodenal ulcer disease patients, after four weeks' treatment with colloidal bismuth subcitrate (240 mg given twice daily). Twenty four hours before the second endoscopy, all patients had stopped colloidal bismuth subcitrate treatment. Multiple biopsy specimens were obtained from the lesser curve of the gastric antrum and duodenal bulb at 5 mm from the ulcer rim. One of the specimens was taken for histological analysis, performed by an observer without knowledge of the biochemical results, and graded as uninfamed (normal) or inflamed (moderate or considerable increase in inflammatory cell infiltrate) using standard histological criteria.18

Department of Surgery,
University College and
Middlesex Hospital
Medical School, The
Middlesex Hospital,
London
A Ahmed
P R Salmon
C R Cairns
M Hobsley
Pharmacology Group,
King's College, London
J R S Hoults

Correspondence to:
Dr A Ahmed, Department of Obstetrics and Gynaecology, University of Cambridge Clinical School, The Rosie Maternity Hospital, Robinson Way, Cambridge CB2 2SW.

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This study was approved by the Clinical Investigation Panel at The Middlesex Hospital and written informed consent was obtained from all subjects.

**PREPARATION AND INCUBATION OF BIOPSY SPECIMENS**

Preparation and incubation procedures were based on those described by Dreyling et al., with minor modifications. The organ culture method in this study employs unstimulated culture for a fixed period followed by a chemically challenged incubation for an identical period. Eicosanoids released during culture were collected from the bathing medium and measured by radioimmunoassay.

Five biopsy specimens for each incubation tube were immediately pooled and washed in 0.5 ml ice cold 0.9% saline. The pooled biopsy specimens (mean (SEM) 18.5 (2.3) mg wet weight antrum and 16.3 (3.2) mg wet weight duodenum) were placed in 0.5 ml 0.9% saline at 4°C for 20 minutes to facilitate the leaching out of any eicosanoids generated during the procedures, and the supernatant was discarded.

After the washout period, the group of five duodenal and antral biopsy specimens was transferred to a similar set of tubes containing 0.5 ml prewarmed and oxygenated (95% O2 + 5% CO2) modified Tyrode solution (composition, g/l: NaCl 0.0, KCl 0.2, MgCl2.6H2O 0.21, NaHCO3 1.0, NaH2PO4, H2O 0.058, CaCl2.2H2O 0.13 glucose 1-1) and incubated in a water bath at 37°C for 20 minutes under continued oxygenation ('basal release').

Biopsy specimens were transferred immediately into a further set of tubes containing fresh prewarmed/oxygenated buffer containing 1 μmol/l of Ca2+ ionophore A23187 (Sigma Chemical Company Ltd). Incubation was continued in the presence of the ionophore for a further 20 minutes at 37°C ('ionophore mediated') and the reaction was terminated by complete removal of the incubation medium. Calcium ionophore A23187 elicits an increase in intracellular Ca2+ which initiates activation of the arachidonic acid cascade. After this incubation the pooled biopsy specimens were removed and weighed and the supernatants from both sets of incubations stored at −20°C. In some experiments (n=8), as indicated, the second incubation was performed in the absence of ionophore A23187.

**RADIOIMMUNOASSAY**

Supernatants from both basal and stimulated release were stored at −20°C for radioimmunoassay – usually within four weeks. Aliquots (25 μl of sample) were assayed in triplicate for PGE2 without extraction using antibodies and double antibody precipitation procedures as described by Berry et al., and the assay sensitivity was 15 pg/tube. LTC4 was measured by a specific LTC4 radioimmunoassay kit (New England Nuclear, DuPont, UK). The antibodies used for determination of the peptide leukotriene had cross reactivities of 100% with LTC4 and 11-6% with LTD4; the assay sensitivity was 20 pg/tube, and the substance(s) detected using this assay are referred to throughout this paper as 'immuno-reactive LTC4'. The intra-assay and the inter-assay coefficients of variation for the PGE2 and LTC4/LTD4 radioimmunoassays were 11.7% and 12.3% (PGE2) and 9.9% and 15.7% (LTC4).

**STATISTICS**

PGE2 and LTC4 values are shown as mean (SEM) expressed as pg/mg wet weight/20 minute incubation (pg/mg/20 minutes). Paired and unpaired data were evaluated using the Student’s t test.

**Results**

**MACROSCOPIC AND HISTOLOGICAL FINDINGS**

In all patients with duodenal ulcer disease, histological examination of the antrum before therapy showed active chronic gastritis with an inflammatory cell infiltrate of predominantly neutrophils and polymorphs. The duodenal tissue showed mild to moderate inflammation in 17 patients, while three patients had normal duodenal histology. Four weeks after treatment with colloidal bismuth subcitrate, gastroscope showed complete ulcer healing in 15/18 patients. Two patients failed to attend for a second gastroscopy. Antral and duodenal histology returned to normal in 18 and 15 patients respectively after treatment. Control subjects had normal antral and duodenal histology as well as being endoscopically normal.

LTC4/PGE2 release in duodenal ulcer disease biopsy specimens before treatment

Immunoreactive PGE2 and LTC4 were released from the biopsy specimens into the bathing media when incubated at 37°C for 20 minutes (basal condition). The mean (SEM) antral basal release of PGE2 was 1438-0 (176-0) pg/mg/20 minutes, and was significantly greater than the mean (SEM) duodenal basal release of 1029-0 (122-0) pg/mg/20 minutes (p<0-05) (Fig 1A). The release of LTC4 was approximately 20 fold less in the antrum and 10 fold less in the duodenum than that of PGE2. The mean (SEM) basal release of LTC4 from antral biopsy specimens was 58-7 (9-5) pg/mg/20 minutes, and was significantly lower than from duodenal specimens (95-5 (13-7) pg/mg/20 minutes (p<0-005) (Fig 1B)).

The generation of LTC4 was increased compared with basal release for both antral and duodenal mucosal tissues when they were transferred for a second incubation with Ca2+ ionophore. Again LTC4 generation was

<table>
<thead>
<tr>
<th>Effect of ionophore A23187 on eicosanoid release. (Values mean (SEM) of eight experiments)</th>
<th>PGE2 (pg/mg/20 min)</th>
<th>LTC4 (pg/mg/20 min)</th>
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<tbody>
<tr>
<td>Antrum</td>
<td>Duodenum</td>
<td>Antrum</td>
</tr>
<tr>
<td>Basal (first incubation)</td>
<td>42 (11-1)</td>
<td>82 (14-2)</td>
</tr>
<tr>
<td>+ A23187 (second incubation)</td>
<td>94 (29-7)</td>
<td>197 (32-4)</td>
</tr>
<tr>
<td>− A23187 (second incubation)</td>
<td>36 (9-8)</td>
<td>65 (12-9)</td>
</tr>
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LTC4 = leukotriene C4; PGE2 = prostaglandin E2.
significantly lower in the antral mucosa than in duodenum, but in both cases output was significantly greater in the A23187 incubation than under basal conditions (antrum: 88.0 (15.2) pg/mg/20 minutes, p<0.05; duodenum: 171.0 (22.7) pg/mg/20 minutes, p<0.01) (Fig 1B).

In contrast, the release of PGE2 from antral and duodenal mucosal biopsy specimens was not stimulated by the ionophore A23187, but was significantly reduced (antrum: 436.0 (8.2) pg/mg/20 minutes (p<0.001); duodenum: 385.0 (69.7) pg/mg/20 minutes (p<0.001)) compared with the basal PGE2 release (Fig 1A).

To examine the effect of A23187 further, experiments were performed comparing the effect of a second incubation with A23187 with that of a second incubation using buffer alone (Table). In this experiment, the amounts of LTC4 and PGE2 and the differences between the antrum and duodenum were very similar to those described above (cf Fig 1). The amount of PGE2 released after addition of the ionophore A23187 did not differ significantly from the amount released during an identical incubation period in the absence of ionophore. The result indicates that PGE2 synthesis seems to be Ca2+ independent in this system. On the other hand and as expected, the release of LTC4 was increased by the calcium ionophore but not in its absence (Table).

The generation of eicosanoids by biopsy samples taken from subjects (n=15) with histologically normal tissue was studied. The release of PGE2 into the bathing medium from normal antral and duodenal biopsy fragments was similar in the order of magnitude and profile to that detected in gastroduodenal mucosa from patients with duodenal ulcer disease (data not shown). The mean (SEM) basal release of LTC4 was 63.6 (9.6) pg/mg/20 minutes from normal antral mucosa and 140.0 (15.1) pg/mg/20 minutes from normal duodenal mucosa. The unstimulated mean LTC4 value from normal antral mucosa was similar in value to the basal antral release from duodenal ulcer disease mucosa (58.7 (9.5) pg/mg/20 minutes), but, surprisingly, the basal duodenal LTC4 release from normal mucosa was significantly (p<0.05) raised compared with the unstimulated release from duodenal ulcer disease mucosa (95.5 (13.7) pg/mg/20 minutes). Analysis of ionophore mediated LTC4 formation showed an interesting difference between normal and abnormal mucosa in the ability of the ionophore to induce LTC4 generation. This was significantly reduced in normal antral (p<0.05) and duodenal (p<0.01) mucosa compared with gastroduodenal mucosa from duodenal ulcer disease patients (Fig 2).

**LTC4/PGE2 RELEASE IN DUODENAL ULCER DISEASE BIOPSY SPECIMENS AFTER TREATMENT**
No significant difference was found in PGE2 release from antral and duodenal biopsy specimens before and after treatment with colloidal bismuth subcitrate (Fig 1A). This was true for comparisons of basal release and for release in the presence of the ionophore.

Analysis of LTC4 formation in biopsy specimens taken from patients after treatment showed considerable differences from the pretreatment values. In antral tissue obtained after completion of colloidal bismuth subcitrate treatment (n=18), there was a significant reduction in basal (p<0.02) but not in ionophore mediated LTC4 release compared with tissue samples taken before treatment (antral basal value 39.0 (5.9) pg/mg/20 minutes; antral stimulated value 74.0 (13.9) pg/mg/20 minutes) (Fig 1B). The basal duodenal LTC4 release was reduced after treatment compared with basal values before therapy (p<0.05) (Fig 1B). More interestingly, the ability of the ionophore to stimulate the release of LTC4 from duodenal mucosa was substantially and significantly reduced after treatment compared with beforehand (p<0.001). The mean ionophore mediated LTC4 value fell from 171.0
The relevance of these findings in rats to chronic relapsing ulcerative disease in man is unclear. Our data showing reduced ionophore mediated release of gastroduodenal mucosa after ulcer healing are consistent with the view that LTC₄ is an important inflammatory mediator, although we imagine that it is probably not the primary agent of injury, but rather a secondary mediator which may amplify mucosal injury. This view is supported by the results shown in Figure 2, which indicate that the ability of the mucosa to generate LTC₄ is greater in duodenal ulcer disease mucosa than in normal mucosa. These results are supported by an earlier report suggesting that normal uninflamed gastric mucosa generates significantly less ionophore mediated LTC₄ than inflamed gastric mucosa.¹

A fundamental problem in assessing the biological importance of eicosanoids is that any perturbation of cell membranes will initiate eicosanoid synthesis, which means that the sampling procedure per se usually stimulates the synthesis and possibly changes the profile of arachidonic acid metabolites. In this study, steps were taken to minimise the effect of sampling trauma by discarding the first collection. The formation of various cyclo-oxygenase products of arachidonic acid metabolism, including PGE₂, by normal and duodenal ulcer disease gastroduodenal mucosa has been described previously.¹² The present study, however, shows important differences between the 5-lipoxygenase and the cyclo-oxygenase pathways in the gastroduodenal mucosal biopsy specimens of duodenal ulcer patients. Under basal conditions, the release of PGE₂ from both the antrum and the duodenum was considerably higher than the release of LTC₄. The observed differences in the levels of these two eicosanoids are probably due to differences in the mucosal cyclo-oxygenase and lipoxygenase enzymatic activity. Human antral and duodenal mucosa generate significantly larger quantities of PGE₂ than LTC₄, probably because of higher cyclo-oxygenase activity in these tissues. Important regional differences in the values of these two eicosanoids were also noted; basal synthesis of PGE₂ was greater in antral mucosal than in duodenal fragments, whereas LTC₄ formation was greater in duodenal than in antral mucosal tissue. These findings are consistent with an earlier report on the prostanoïd profile in the human gastrointestinal tract.¹³

The calcium ionophore A23187 stimulated mucosal LTC₄ formation but had no effect on mucosal PGE₂ synthesis. It has been suggested that the prostaglandins and leukotrienes that can be shown to be released from specimens of mucosal tissue may come from different cellular sources.¹⁴ It is also possible that the inability of calcium ionophore to stimulate mucosal PGE₂ formation may be related to a Ca²⁺ independent phospholipase A₂ as the rate limiting enzyme that catalyses the hydrolysis of phospholipids containing arachidonic acid. Several recent studies have raised the possibility that phospholipase A₂ activation also involves Ca²⁺ independent mechanisms.² The presence of a Ca²⁺-
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independent phospholipase A2 with a substrate specificity for alkyl containing glycerophospholipids was shown in fetal rabbit lung tissue and human amnion tissue. It has also been shown that a phosphatidylethanolamine specific phospholipase A2 purified from human platelets does not require Ca2+ for activity.

Colloidal bismuth subcitrate was found to be effective in healing duodenal ulcers, and this is consistent with previous reports.6,7 In contrast with earlier workers,9,10 who showed increased PGE2 synthesis after intragastric administration of colloidal bismuth subcitrate in rats, however, no change in the PGE2 profile was found before and after colloidal bismuth subcitrate treatment. One possible explanation for this difference may be that in the acute ethanol induced rat model, topical application of colloidal bismuth subcitrate may act as a mild stimulant, thus generating endogenous PGE2 via the mechanism commonly known as adaptive cytoprotection. No such mechanism, however, would be operative in chronic duodenal ulcer disease.

In this study, treatment of duodenal ulcers with colloidal bismuth subcitrate was associated with the clearance of inflammatory cell infiltrate as well as with a decrease in synthesis of LTC4 after ionophore stimulation. These results suggest that decreased LTC4 formation is probably a secondary event caused by the reduction in the numbers of inflammatory cells that the peptidoleukotrienes are probably not the primary agents of injury but may play a role in the development ulceration.